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EFFECT OF STAPHYLOCOCCAL ENTEROTOXIN B ON CARDIORENAL FUNCTIONS--ETC(U)  
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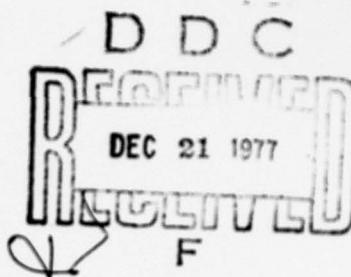
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Effect of Staphylococcal Enterotoxin B  
on Cardiorenal Functions and Survival  
in X-Irradiated Rhesus Macaques\*

C. T. Liu, Ph.D., M. J. Griffin, and D. E. Hilmas, D.V.M., Ph.D.

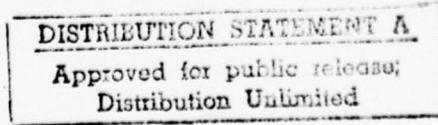
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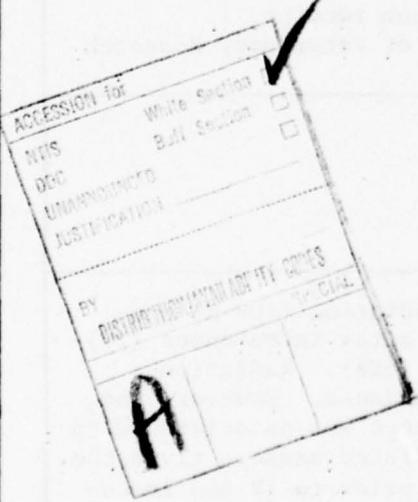
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(where measured at 5 hours) in cardiac output, stroke volume,  $T_{H_2O}^C$ ,  $C_{PAH}$ ,  $C_{osm}$ , and urine flow, as well as increases in total peripheral and renal resistance. A theory concerning the significance of radiation-induced leukopenia on modification of SEB-induced cardiorenal functions is postulated.



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## Summary

### Pretreatment of monkeys with nonlethal total-body x-irradiation

R) prolonged survival time from an average of 15 to 101 hours after intravenous (IV) inoculation of 50  $\mu\text{g}/\text{kg}$  of staphylococcal enterotoxin B (SEB). Radiation exposure per se did not produce detectable cardiorenal changes. However, the longer survival after SEB challenge in x-irradiated monkeys was associated with improved cardiorenal functions when compared to nonirradiated monkeys given the same dose of SEB. Total-body radiation exposure 4 days prior to IV SEB inoculation prevented typical SEB-induced decreases (where measured at 5 hours) in cardiac output, stroke volume,  $\frac{\text{Tc}}{\text{H}_2\text{O}}$ ,  $\frac{\text{C}_{\text{PAH}}}{\text{C}_{\text{osm}}}$ , and urine flow, as well as increases in total peripheral and renal resistance. A theory concerning the significance of radiation-induced leukopenia on modification of SEB-induced cardiorenal functions is postulated.

\* concentration of solute-free water,  
\*\* clearance of para-amino-hippurate,  
\*\*\* osmolar clearance,

### Introduction

Previous studies have shown that after intravenous (IV) injection of a lethal dose of highly purified staphylococcal enterotoxin B (SEB) to antibody-negative rhesus macaques, cardiovascular, hepatic, and renal functions were impaired and death occurred within 10 to 20 hours.<sup>1-3</sup> The exact cause of SEB-induced death is unknown, even though numerous theories including dehydration,<sup>4</sup> cardiac and pulmonary dysfunctions,<sup>2,5</sup> and peripheral capillary pooling<sup>6,7</sup> have been proposed. Others have found that leukopenia and accumulations of leukocytes were present in the edematous lung during SEB enterotoxemia in monkeys.<sup>8,9</sup> Furthermore, Crawley et al<sup>10</sup> and Stiles and Denniston<sup>9</sup> postulated that leukocytes bind with SEB in the lung to play an important role for increasing capillary permeability.

Total-body x-irradiation has been shown to alleviate endotoxemia and prolong survival in dogs and mice.<sup>11-13</sup> Although the mechanism for this protection has not been determined, it appears that x-irradiation modifies the sensitivity of the host to bacterial endotoxin challenge. The main objective of this study was to examine whether pretreatment of macaques with nonlethal total body x-irradiation would prolong survival and alter cardiorenal responses to IV inoculation with a lethal dose of SEB.

### Methods and Materials

A total of 29 female rhesus macaques, weighing 3.5 to 5.5 kg, with negative SEB antibody titers were used. The organization of experiments and number of macaques allocated in each group are presented in Table 1. Since comparable cardiorenal data for groups I (control) and II (SEB-inoculated macaques) have been reported from this laboratory,<sup>1,3</sup> values obtained from groups III (x-irradiated) and IV (x-irradiation + SEB) are new information.

Techniques for measuring various cardiovascular and renal functions including mean arterial blood pressure, cardiac output, stroke volume, total peripheral resistance, clearance of insulin ( $C_{in}$ ), clearance of para-amino-hippurate ( $C_{PAH}$ ), osmolar clearance ( $C_{osm}$ ), tubular concentration of solute-free water ( $T_{H_2O}^C$ ), renal blood flow, and renal vascular resistance of conscious rhesus macaques have been described previously.<sup>14</sup> An IV dose (50 µg/kg) of highly purified SEB<sup>15</sup> was administered to group II nonirradiated macaques and to group IV macaques 4 days after a single exposure to sublethal total-body x-irradiation (400 R).

The total-body x-radiation exposure dose (400 R) was delivered from a 1 MeV, 3 mA, x-ray generating unit \*\* operating at an effective energy of 475 keV without added filtration. Before irradiation, each monkey was anesthetized with Ketamine (40 mg/kg, intramuscularly) and placed in a Lucite cylinder (length = 49.5 cm; diameter = 13.6 cm) which was rotated at 1 rpm in the x-ray beam. The midline exposure dose rate was 30 R/minute in air at a distance of 118 cm.

All data were analyzed statistically. When values were compared from the same macaque between the base line and later values of any

group, the paired t-test was employed. When comparisons were made between 2 groups, i.e., control vs. SEB, control vs. x-irradiated, or x-irradiated vs. x-irradiated + SEB, the mean changes in values from each macaque's own control value for a particular time interval were compared between groups using an independent t test. The "null" hypothesis was rejected at the 5% level.

### Results

Cardiovascular Responses--A gradual decrease in mean blood pressure occurred within 5 hours of injection of SEB in both the irradiated and nonirradiated monkeys (Fig. 1). Unlike nonirradiated monkeys, however, the irradiated group failed to develop a significant decrease in cardiac output and stroke volume or an increase in total peripheral resistance at 5 hours (Figs. 2-4). Total-body x-irradiation with 400 R did not alter significantly any of the measured cardiovascular variables.

Renal Responses--Typical renal responses to SEB intoxication in nonirradiated macaques (group II) included a gradual but significantly increased renal vascular resistance and decreased  $C_{in}$ ,  $C_{PAH}$ , total renal blood flow, urine flow,  $C_{osm}$ , and  $T_{H_2O}^C$  as shown in Figures 5-11. In contrast, these were not significantly different from control values at 5 hours in irradiated monkeys given the dose of SEB. No significant renal changes were observed in the irradiated control macaques 4 days after a 400 R exposure dose.

Survival Time--The effect of pretreatment with sublethal irradiation on survival following a lethal challenge dose of SEB is shown in Table 2. The mean survival time of  $101 \pm 24$  hours (mean  $\pm$  SE) for x-irradiated macaques was markedly longer than that of nonirradiated control group

(5 to 20 hours) given the same dose of SEB. Even though survival time was prolonged, none of the irradiated macaques inoculated with SEB survived indefinitely.

#### Discussion

These experiments showed that pretreatment with sublethal total-body x-irradiation exposure (400 R) diminished the changes in selected physiologic functions observed in the nonirradiated SEB-intoxicated macaques and enhanced the resistance of the host to this toxicity.

The evidence was based on a diminution in characteristic 5-hour cardiorenal responses associated with SEB toxemia and a significantly prolonged survival time. These data were also in agreement with the observation that intact irradiated rabbits survived inoculation with an otherwise lethal dose of SEB.<sup>16</sup> Similarly, total-body x-irradiation of dogs or mice resulted in significant protection against endotoxemia.<sup>11-13</sup>

Identification of radiation-induced modifications of the host that prevent development of fatal toxemia may serve as a guide in the search for effective methods of treatment.

Although pretreatment with total-body x-irradiation protected monkeys against both the relatively rapid onset of cardiorenal dysfunction and early death following IV SEB inoculation, all irradiated challenged monkeys died within 3 to 5 days. It is possible, however, these deaths were the result of bacterial infection associated with experimental procedures, i.e., abdominal surgery without antibiotic treatment. Although superimposed bacterial infections were not confirmed in these macaques, the leukopenic response to SEB superimposed on irradiation-induced leukopenia could have interfered with mobilization

of defenses against infections caused by surgical trauma, the enterotoxemia, or some combination of both stresses.

In our previous studies with rabbits,<sup>16</sup> decreased O<sub>2</sub> consumption and leukopenia reached minimum values 4 days after irradiation.

Hypometabolism and its associated decrease in tissue O<sub>2</sub> demand were considered as a possible mechanism for increased tolerance to SEB challenge. However, this theory could not be substantiated, because hypometabolism in rabbits, induced by thyroidectomy, starvation, or water deprivation, failed to cause any significant change in death time after SEB inoculation. Therefore, factors other than decreased O<sub>2</sub> consumption are associated with the early prevention of SEB-induced cardiorenal dysfunctions in the irradiated host. Our attention has been directed to the role of leukocytes.

Intravenous injection of SEB in microgram quantities has been shown to produce shock and death in rhesus macaques.<sup>17</sup> Rapoport et al<sup>18</sup> observed that 80 to 90% of IV injected SEB disappeared rapidly from the circulation within 30 minutes. The major sites for SEB accumulation included the renal cortex, liver, and lung.<sup>19</sup> Among these target organs,<sup>1-3,5</sup> it appears that impairment of lung functions and pulmonary edema are the direct cause of death. Since bone marrow depression and leukopenia occur a few days after total-body x-irradiation in monkeys,<sup>20,21</sup> a possible relationship between radiation-induced leukopenia and the decrease in SEB toxicity is postulated. This is supported by evidence that SEB in the circulation is not transported by plasma protein,<sup>22</sup> but rather is bound to leukocytes.<sup>10</sup> Leukocytes may serve as "carriers" to mobilize SEB from various tissues for subsequent circulation to the lungs, where the leukocytes accumulate following inoculation with an IV dose of SEB.<sup>8,9</sup> Further, the presence of a

large number of leukocytes in the lung may contribute to further pulmonary dysfunction<sup>23</sup> and enhance the formation of kinins<sup>24</sup> to increase pulmonary capillary permeability. In the leukopenic state as induced by irradiation, it is possible that the quantity of SEB-transport "carriers" (leukocytes) is decreased and the amount of kinin formation is simultaneously reduced. Consequently, SEB-induced pulmonary edema is prevented, decreased in severity or develops more gradually.

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Footnotes

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\*\* General Electric Co., Milwaukee, Wis.

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**TABLE 1. Allocation of 29 Rhesus Macaques into Groups**

Group	Classification	No. of Macaques
I	Control	10
II	SEB	8
III	X-irradiated	4
IV	X-irradiated + SEB	7

TABLE 2. Effect of Pretreatment with Total-body X-irradiation (400 R) on Survival during Type B Enterotoxemia in Rhesus Macaques

Group	No	Dose of SEB ( $\mu$ g/kg)	Survival Time (hours)	No. Dead/Total
X-irradiated	4	None	Indefinite	0/4
SEB	8	50	5-20	8/8
X-irradiated + SEB	7	50	101 $\pm$ 24	7/7

\*SEB was given 4 days after exposure to 400 R of total-body x-irradiation.

**FIGURE LEGENDS**

Fig. 1--Effect of IV SEB (50 µg/kg) on mean blood pressure in control and irradiated monkeys.

Fig. 2--Effect of IV SEB (50 µg/kg) on cardiac output in control and irradiated monkeys. \*  
p < 0.05.

Fig. 3--Effect of IV SEB (50 µg/kg) on stroke volume in control and irradiated monkeys. \*  
p < 0.05.

Fig. 4--Effect of IV SEB (50 µg/kg) on total peripheral resistance in control and irradiated monkeys. \*  
p < 0.05.

Fig. 5--Effect of IV SEB (50 µg/kg) on renal vascular resistance in control and irradiated monkeys. \*  
p < 0.05.

Fig. 6--Effect of IV SEB (50 µg/kg) on clearance of inulin (glomerular filtration rate) in control and irradiated monkeys. \*  
p < 0.05.

Fig. 7--Effect of IV SEB (50 µg/kg) on clearance of para-aminohippurate (effective renal plasma flow) in control and irradiated monkeys.

\* p < 0.05, \*\* p < 0.01.

Fig. 8--Effect of IV SEB (50 µg/kg) on total renal blood flow in control and irradiated monkeys. \*  
p < 0.05.

Fig. 9--Effect of IV SEB (50 µg/kg) on urine flow rate in control and irradiated monkeys. \* $p < 0.05$ , \*\* $p < 0.01$ .

Fig. 10--Effect of IV SEB (50 µg/kg) on osmolal clearance in control and irradiated monkeys. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Fig. 11--Effect of IV SEB (50 µg/kg) on renal tubular concentration of solute-free water in control and irradiated monkeys: \* $p < 0.05$ , \*\* $p < 0.01$ .

Control (9)

SEB (8)

140

120

100

$\frac{d}{H}$  E E

X-Irradiated (4)

X-Irradiated + SEB (7)

140

120

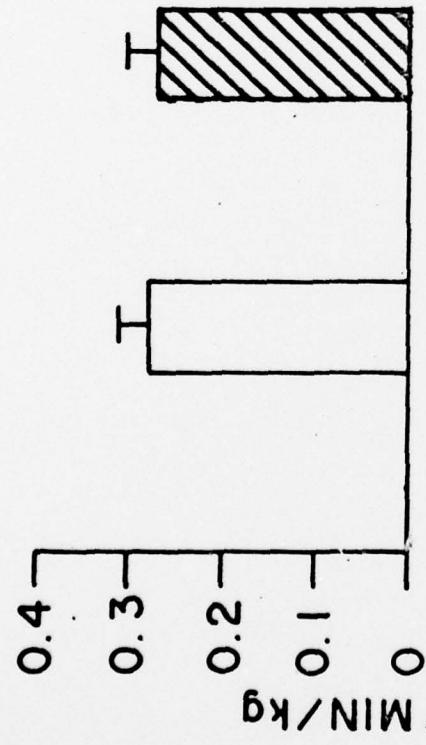
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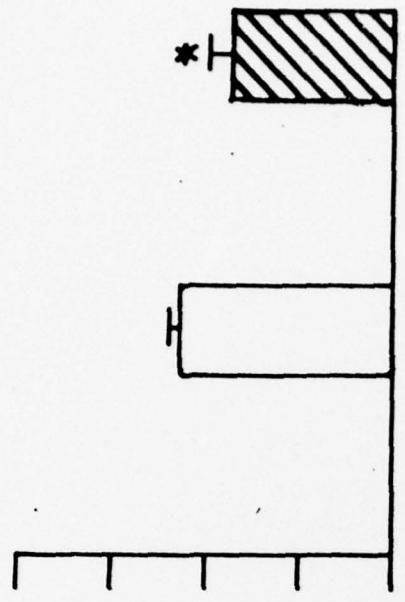
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HOURS

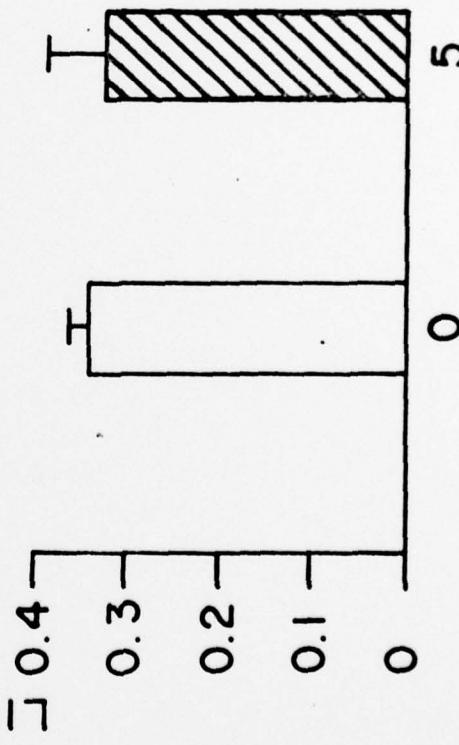
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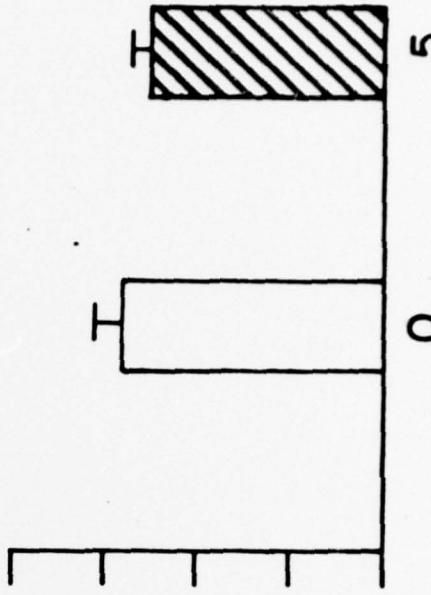
SEB (7)



X-IRRADIATED (4)

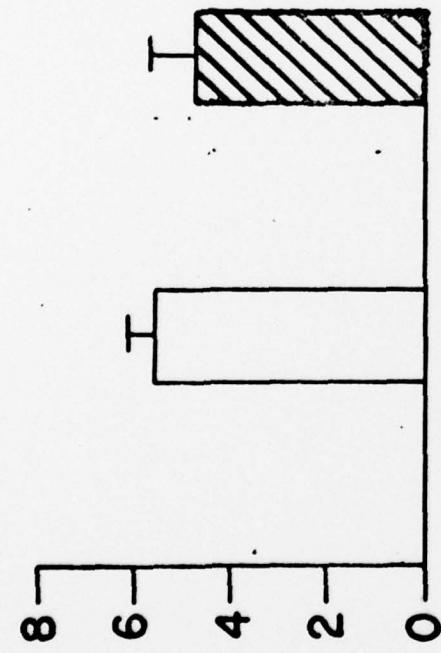


X-IRRADIATED + SEB (7)

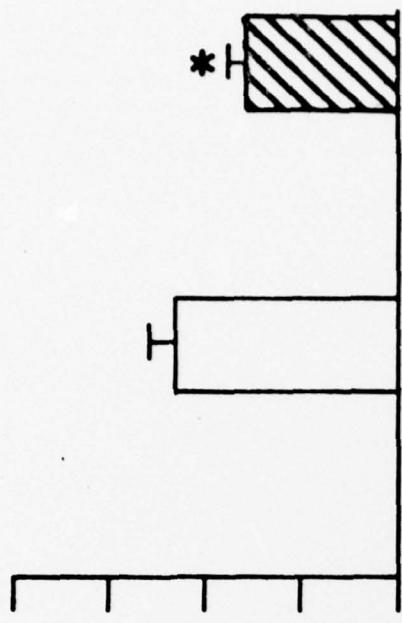


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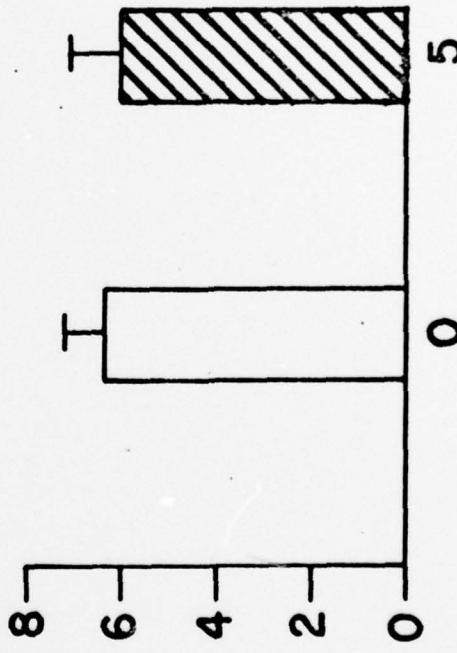
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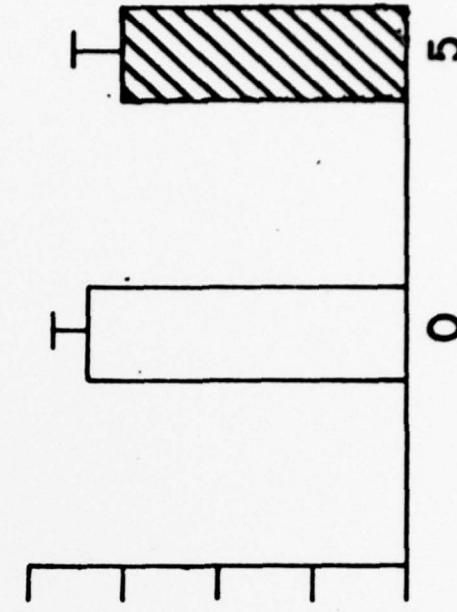
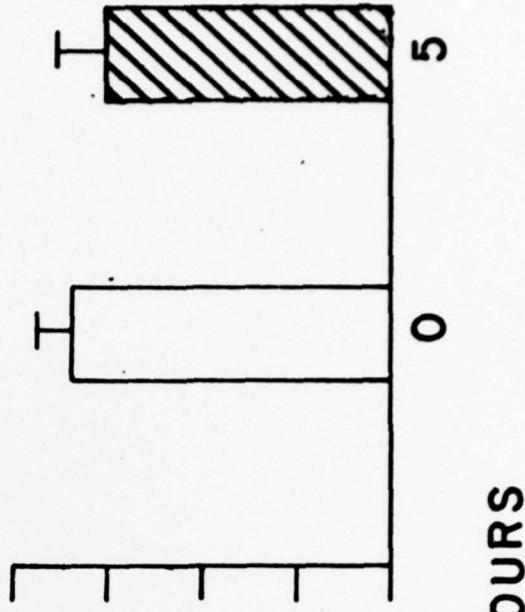
SEB (7)



X-IRRADIATED (4)



X-IRRADIATED + SEB (7)



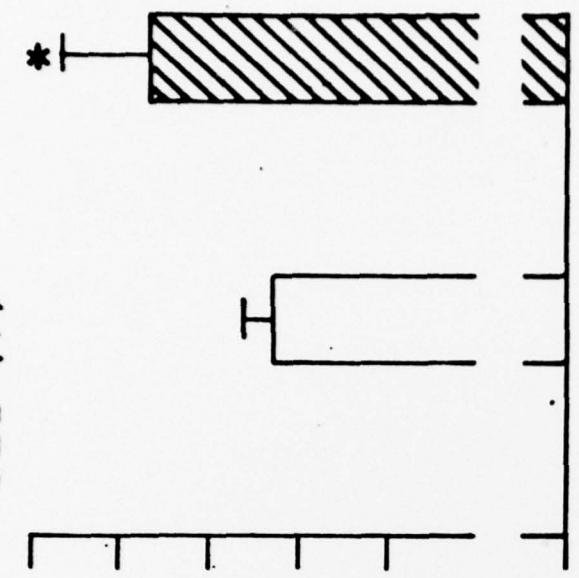
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CONTROL (6)

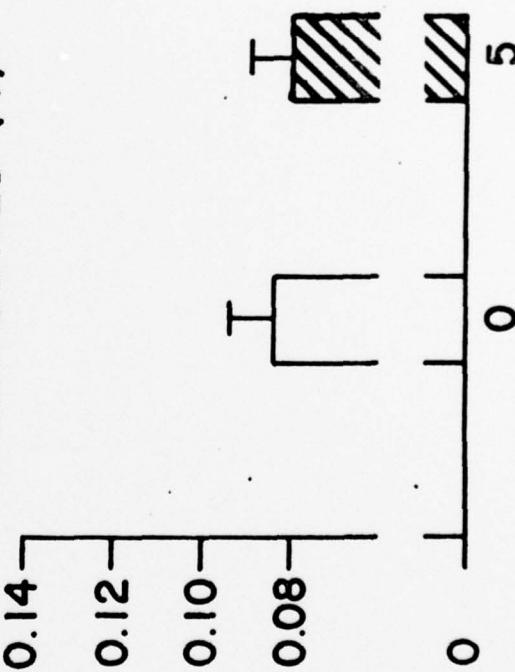


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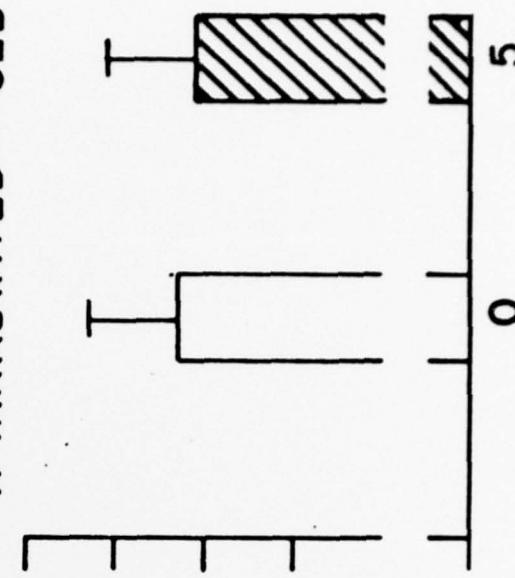


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X-IRRADIATED (4)



X-IRRADIATED + SEB (7)



HOURS

5

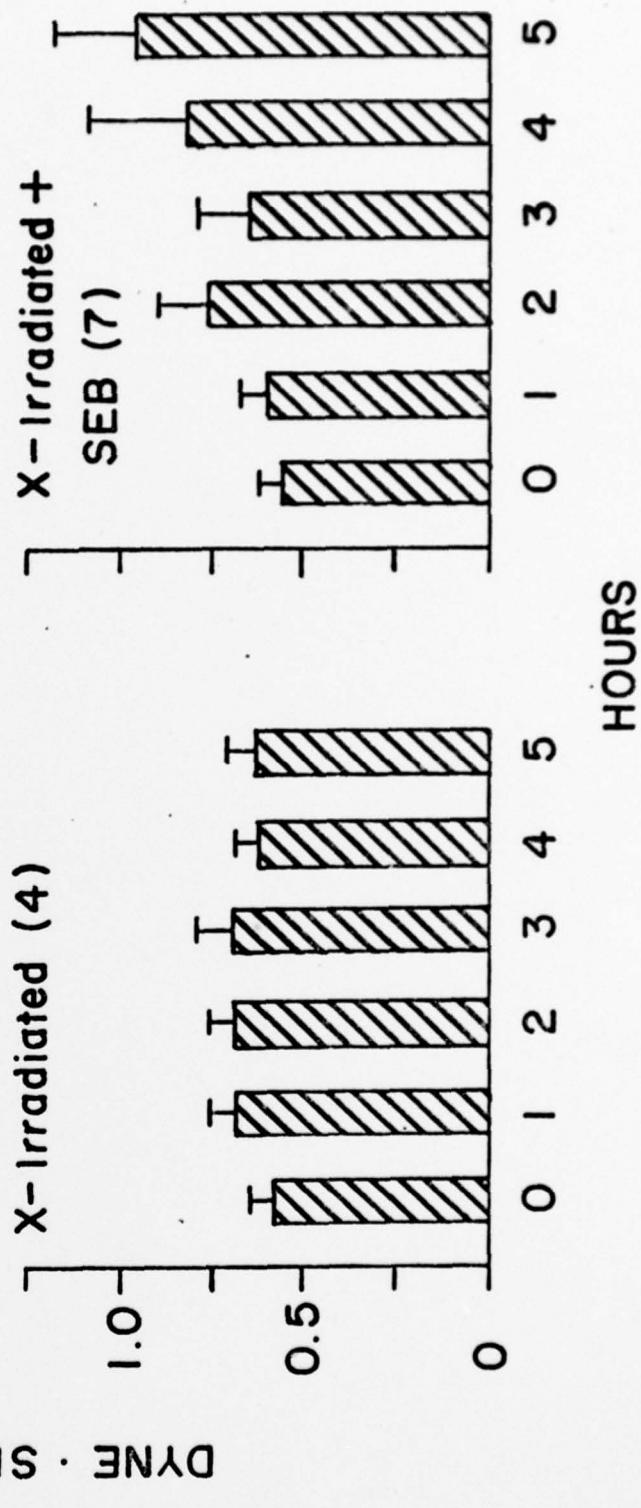
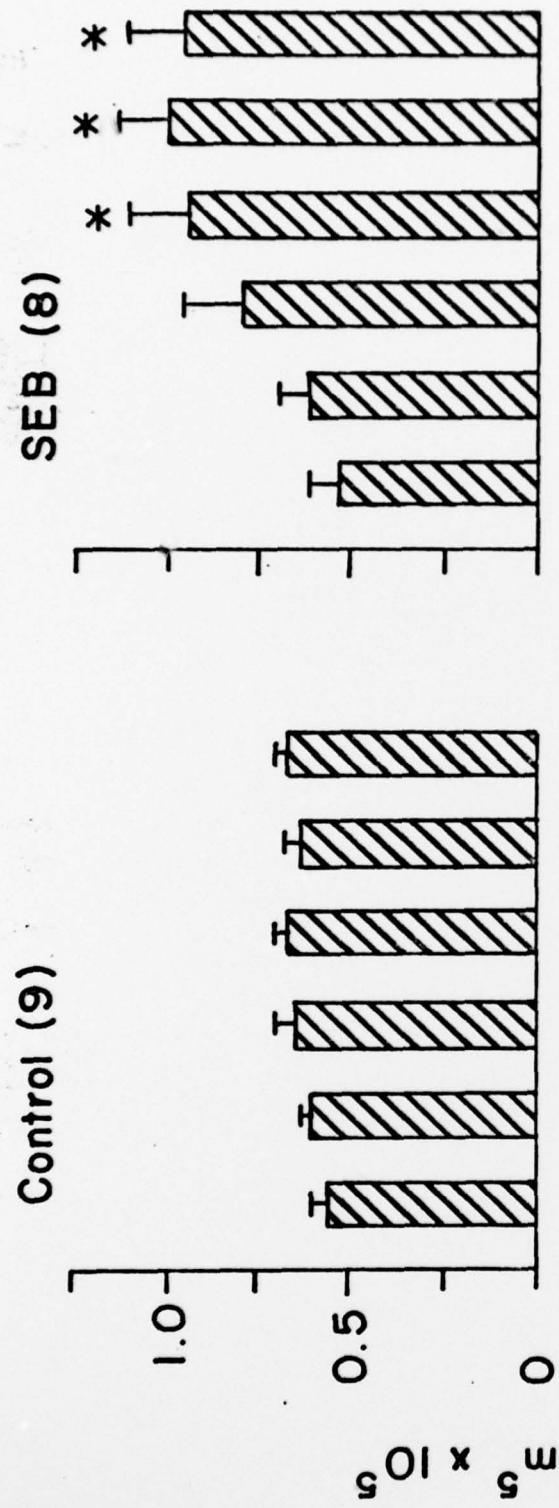
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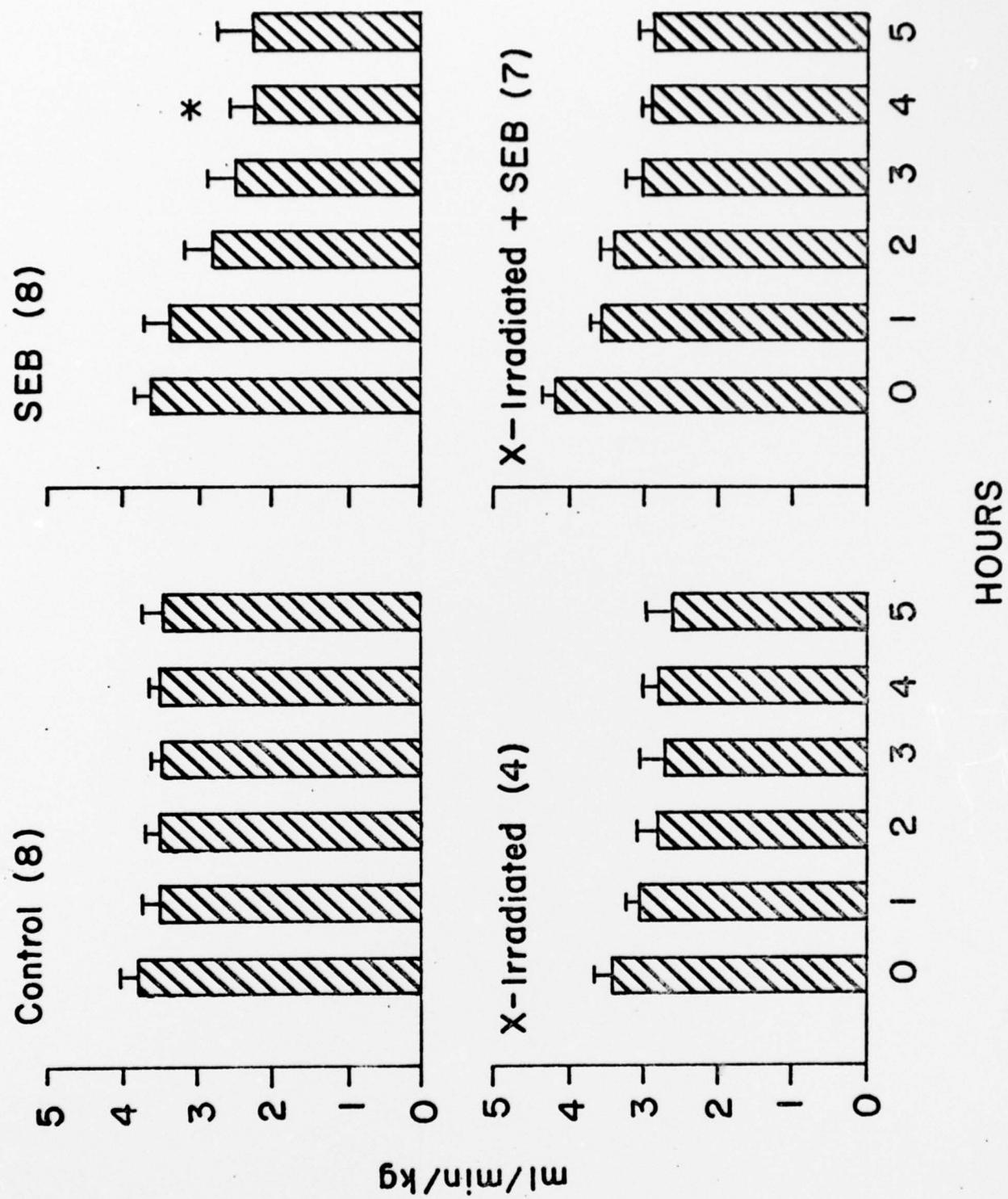
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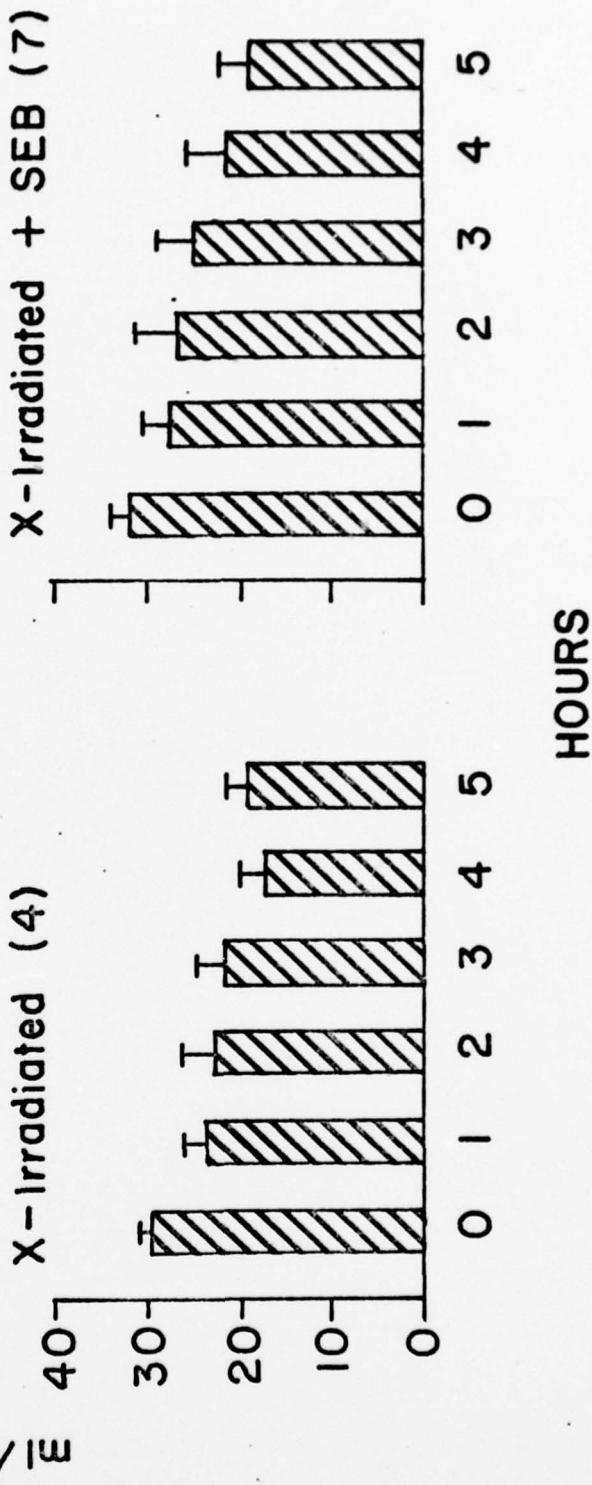
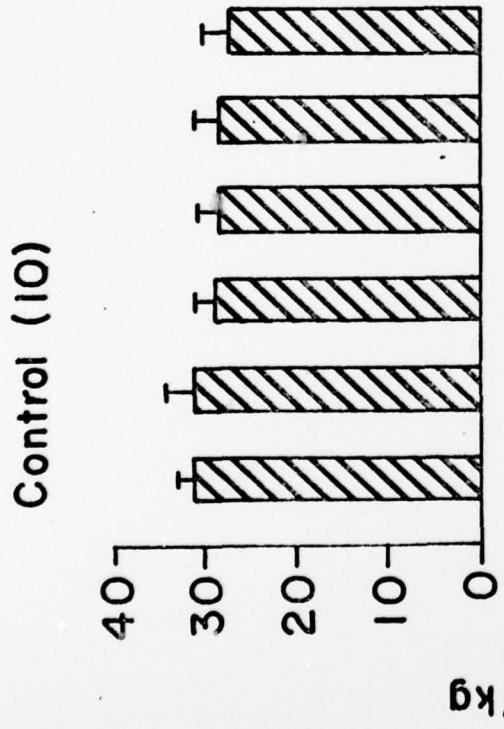
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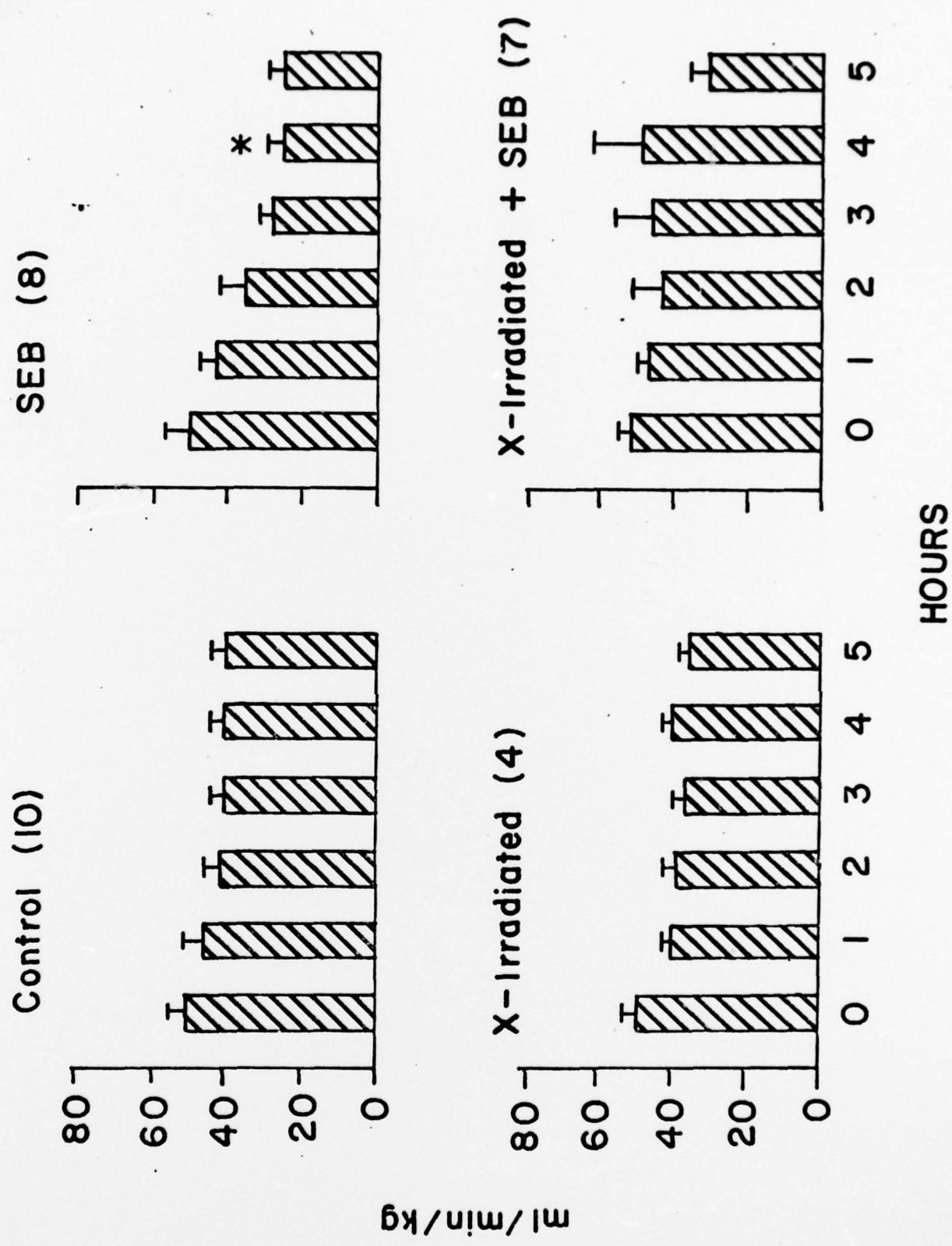
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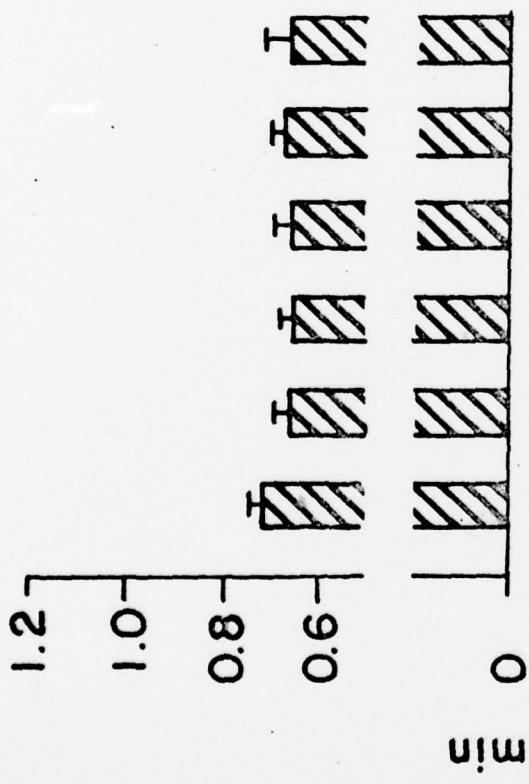




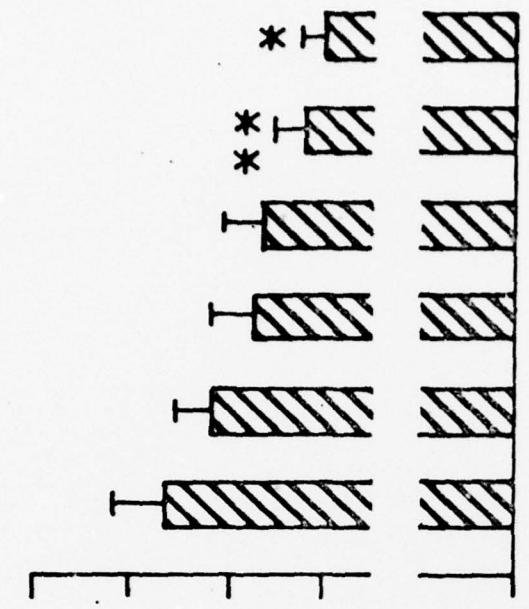




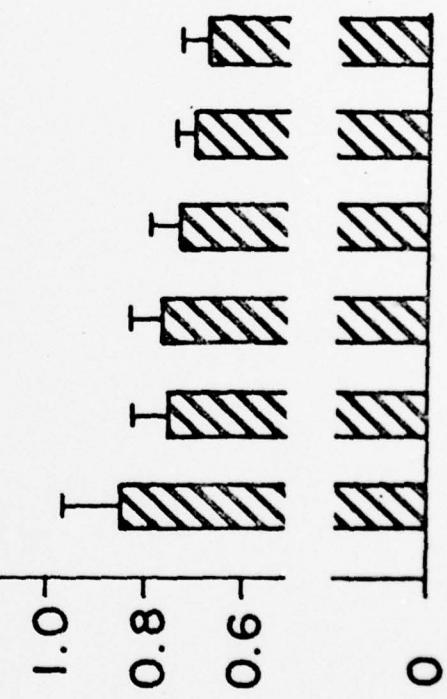
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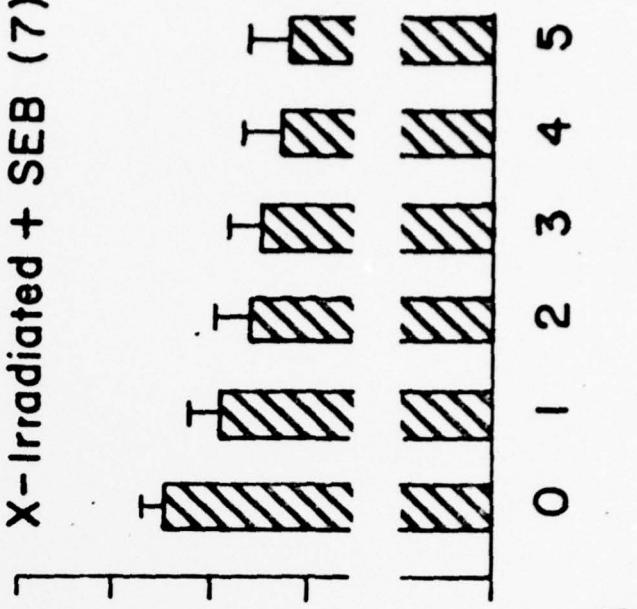
**SEB (8)**



**X-Irradiated (4)**



**X-Irradiated + SEB (7)**



**HOURS**

